Classification and correlation of *RYR2* missense variants in individuals with catecholaminergic polymorphic ventricular tachycardia reveals phenotypic relationships

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Abstract

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is predominantly caused by heterozygous missense variants in the cardiac ryanodine receptor, *RYR2*. However, many *RYR2* missense variants are classified as variants of uncertain significance (VUS). We systematically re-evaluated all *RYR2* variants in healthy individuals and those with CPVT or arrhythmia using the 2015 American College of Medical Genomics guidelines. *RYR2* variants were identified by the NW Genomic Laboratory Hub, from the published literature and databases of sequence variants. Each variant was assessed based on minor allele frequencies, *in silico* prediction tools and appraisal of functional studies and classified according to the ACMG-AMP guidelines. Phenotype data was collated where available. Of the 326 identified *RYR2* missense variants, 55 (16.9%), previously disease-associated variants were re-classified as benign. Application of the gnomAD database of >140,000 controls allowed reclassification of 11 variants more than the ExAC database. CPVT-associated *RYR2* variants clustered predominantly between amino acid positions 3949-4332 and 4867-4967 as well as the RyR and IP3R homology associated and ion transport domains ($P < 0.005$). CPVT-associated *RYR2* variants occurred at more conserved amino acid positions compared to controls, and variants associated with sudden death had higher conservation scores ($P < 0.005$). There were five potentially pathogenic *RYR2* variants associated with sudden death during sleep which were located almost exclusively in the C-terminus of the protein. In conclusion, control sequence databases facilitate reclassification of *RYR2* variants but the majority remain as VUS. Notably, pathogenic variants in *RYR2* are associated with death in sleep.

KEYWORDS: Catecholaminergic ventricular tachycardia, cardiac ryanodine receptor, variant classification, arrhythmia.

Introduction

The rare monogenic arrhythmogenic disorder catecholaminergic polymorphic ventricular tachycardia (CPVT, MIM 604772) is characterised by episodic ventricular dysrhythmia triggered by exercise or emotion in individuals without structural cardiac defects (1). CPVT can be inherited in both an autosomal dominant form caused by heterozygous pathogenic variants in the cardiac ryanodine receptor gene (*RYR2*) (MIM 180902)(2), less frequently in *CALM1* (MIM 114180)(3) encoding calmodulin 1 and autosomal recessive form due to biallelic variants in *CASQ2* (MIM 114251)(4) encoding calsequestrin, *TRDN* (MIM 603283)(5) encoding triadin and *TECRL* (MIM 617242)(6). Furthermore, CPVT can also be caused by rare deletions in exon 3 of *RYR2* (7). However, in such cases CPVT is often accompanied by left ventricular non-compaction (7). It is estimated that one in 10,000 people are clinically affected by the condition, with sudden cardiac death being the first manifestation in some individuals (1, 8, 9). The phenotypic heterogeneity of CPVT can delay or obscure diagnosis. It has been reported that almost one in three individuals with CPVT are initially diagnosed with long QT syndrome (LQTS) despite a normal QT interval (2). Consequently, combined approaches of cardiac assessment including exercise stress testing and genetic analysis are used to confirm a diagnosis of CPVT.

The large coding region of the cardiac ryanodine receptor (105 exons) previously made genetic testing costly and time consuming using conventional DNA sequencing methods. As a result, it became common practice to only screen the four regions considered to be mutation hotspots in *RYR2* (10). Next generation sequencing has now become more widely available and all coding exons of *RYR2* can be screened rapidly and cheaply. This has led to an increase in the number of *RYR2* variants being reported in individuals with cardiac dysrhythmia or associated symptoms of palpitations, syncope or sudden unexplained death. Concomitant with this has been the increase in *RYR2* variants identified in apparently healthy individuals collated through international resources, including the Genome Aggregation Database (gnomAD)(11). The majority of *RYR2* variants are

missense changes. When assayed in functional experiments a number of these lead to increased channel activity consistent with pathogenicity. However, the rarity of these variants and complexity of functional assays makes it difficult to determine their pathogenicity, and so the majority are classified as variants of uncertain significance (VUS). Recently the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) proposed guidelines to standardise the classification of genetic variants (12).

In this study we collate *RYR2* variants reported in individuals with, or suspected of having CPVT, and classify them according to these ACMG guidelines and correlate the variants with clinical features.

Methods

A comprehensive search for *RYR2* variants identified in individuals undergoing genetic testing for CPVT or an associated arrhythmia was performed. A total of 326 different *RYR2* variants was obtained from the North West Genomic Laboratory Hub, UK (a service that has been undertaking clinical diagnostic testing of RYR2 for >10 years), the published literature and clinical variant databases, including ClinVar and the Human Gene Mutation Database (HGMD) (Supplementary Table 1) (13, 14). Allele frequencies of *RYR2* variants in apparently healthy individuals were obtained from gnomAD as a comparator, accessed online from https://gnomad.broadinstitute.org (11).

Phenotype-Genotype analysis

All *RYR2* variants both in control and CPVT populations were mapped to the domains, structural motifs and regions in which they are located in the RyR2 protein (using the universal protein resource (UniProt) accession number Q92736) with Mutation Mapper from the cBio Cancer Genomics Portal. For the purpose of our analysis *RYR2* regions that were not associated with any known functional or structural domains were individually labelled as 'no domain' followed by a number ranging from 1 to 9 corresponding to their location. The proportion of missense variants in each region/domain of *RYR2* in control and CPVT populations were compared using the Fisher's

exact test using GraphPad prism. To account for multiple testing a p-value < 0.005 was considered significant.

Variant Classification

RYR2 variants were classified based on the 2015 ACMG-AMP guidelines. As reported by Denham *et al.* (2019) 12 of the 27 criteria listed in the ACMG-AMP guidelines were excluded from this study as they were considered non-applicable (reasons for the exclusion of these criteria are included in Supplementary Table 2) (15). The application of these guidelines has been previously reported (Supplementary Table 3 and 4)(15).

Criteria for segregation

The criteria for a variant to qualify for variant segregation (PP1) required that the variant was present in two or more members of the same family with a CPVT-like phenotype (arrhythmias, syncope, bradycardia or sudden death). The occurrence of affected individuals in whom the putative variant did not segregate was considered strong evidence for a benign classification (BS4).

Criteria for functional studies

Robust functional studies, including animal models, calcium imaging, cellular electrophysiology and single channel analysis showing either a significant reduction or gain of function, were considered strong evidence for pathogenicity (PS3). Conflicting functional data on variants was not considered as positive evidence of pathogenicity. Functional studies that reported no change in channel function were considered evidence for a benign classification (BS3).

Criteria for variant frequency

Variants that were absent from gnomAD were considered ultra-rare (PM2) and variants with an allele count of below 4 were considered rare.

The statistical framework used to identify variants that occur too frequently in the gnomAD database to be pathogenic has been described by Whiffin et al. (2017) (16). To summarize, it involves the determination of the maximum credible population allele frequency for a missense variant in *RYR2* that causes CPVT. This was calculated based on CPVT as a dominant condition with a penetrance of approximately 60% (8). A binomial distribution of the maximum credible allele frequency was generated for our sample of CPVT cases (observed allele number) and the upper boundary of the 95% confidence interval (the maximum tolerated allele count) was used as the cut off frequency. Variants that occurred more frequently than the maximum tolerated allele count in gnomAD were considered common and this was strong evidence for a benign classification (BS1).

Criteria for variant enrichment in CPVT cases

Ultra-rare and rare variants were considered for variant enrichment analysis. The presence of an ultra-rare or rare variant in at least five or ten CPVT cases, respectively, was considered as strong evidence (PS4).

Criteria for computational evidence

To remain consistent with previously reported variant classification methods, five protein-level in silico prediction tools: SIFT, PolyPhen, Mutation Taster, Mutation assessor, FATHMM and three conservation tools GERP++, PhyloP conservation and SiPhy were used for the computational analysis of variants where DNA positional information was provided (GERP++, PhyloP, and SiPhy scores of 4.4, 1.6, and 12.17, respectively were set as thresholds for conservation)(15). In addition, Consurf (http://consurf.tau.ac.il/), which uses advanced probabilistic evolutionary models to distinguish true conservation resulting from purifying selection and produces estimates for the credibility of the results, was used to measure conservation of amino acid positions of variants in controls and CPVT patients. The Consurf scores of the amino acid positions of control, CPVT and sudden death cases were compared using the Mann-Whitney test using GraphPad prism, p-value <0.005 was considered significant.

Criteria for critical functional domain

The location of a missense variant in the transmembrane 4-6 region or ion-transport domain of the protein was considered as moderate evidence for a pathogenic classification, as the functional significance of these regions has been established (17, 18).

Results

Collation of RYR2 missense variants

A total of 326 independent *RYR2* single nucleotide, non-synonymous variants associated with CPVT or arrhythmia were identified. Of these variants 97 were present in both control and CPVT populations. Importantly, 104 (31.9%) of the CPVT-associated *RYR2* variants were located outside of regions previously considered mutation hotspots. The hotspot regions with the most *RYR2* variants were domains III and IV, where 21.5% and 21.2% of variants were located, respectively. The numbers of male and female cases were similar (Table 1).

The most common amino acid position at which missense changes occurred was Arg420. Nineteen (19) of 440 (4.3%) independent cases were reported to have a missense change at this amino acid position, 10 cases carried the Arg420Trp variant and 9 carried the Arg420Gln variant. The second most common protein position for missense changes was Arg176, 8 of 440 (1.8%) cases carried the Arg176Gln variant.

Genotype-Phenotype analysis

Several domains or regions within *RYR2* contained a significantly higher proportion of CPVT-associated missense variants compared with controls (Figure 1 and Table 2). CPVT-associated missense variants occurred more frequently than expected between amino acid positions 3949-4332 and 4867-4967 (No Domain regions 5 and 7). CPVT-associated *RYR2* variants were also enriched in the RyR and IP3R homology-associated and ion transport domains when compared to control variants ($p < 0.005$). In contrast, control variants clustered between amino acid positions 2906-3826

and the SPRY and RYR domain when compared to CPVT-associated variants ($p < 0.005$). There was no clear relationship between sudden death and the location of CPVT-associated *RYR2* variants (Figure 1A, Supplementary Table 5). However, five of the nine *RYR2* variants associated with sudden death during sleep occurred in the C-terminus of the protein (Figure 1A, Supplementary Table 6).

Conservation analysis

CPVT variants occurred at positions with significantly higher Consurf scores than controls ($P < 0.0001$), and variants identified in individuals or families with a history of sudden death had higher Consurf scores compared to variants in individuals and families without a history of sudden death ($P < 0.0001$) (Figure 2). This suggests variants with Consurf scores above 7 are more likely to be CPVT-associated and of these variants those with Consurf scores above 8 are more likely to be associated with sudden death.

Classification of RYR2 variants

RYR2 variants were classified according to the ACMG-AMP guidelines and statistical methods were used to identify those variants that occurred too frequently in controls to be pathogenic, these variants were classified as benign (Supplementary Table 7). Using the statistical method described by Whiffin et al. (2017)(16), data on the most common CPVT-associated *RYR2* variant c.1258C>T (Arg420Trp) and control populations from the ExAC or gnomAD databases, the maximum tolerated allele count for CPVT associated *RYR2* variants was calculated (Supplementary Tables 8 and 9).

The maximum tolerated allele count for pathogenic *RYR2* variants was calculated to be two when using the ExAC database as a control population and three for the gnomAD database. Using the gnomAD database and the maximum tolerated allele count as a frequency threshold for pathogenicity 55 of 326 previously putative disease associated variants were re-classified as benign according to the ACMG guidelines, 11 fewer variants (44) were reclassified as benign using the ExAC database. A further 245 variants were classified as variants of uncertain significance, 14 as likely

pathogenic and 12 as pathogenic using gnomAD as the control comparator (Table 3). Both benign and pathogenic variants occurred most frequently outside of known functional domains. The ion transport domain contained the most (7 of the 26) pathogenic or likely pathogenic variants. The SPRY domain was found to be the domain containing the most benign variants; this domain did not contain any pathogenic variants.

Sufficient functional data to aid classification was available for 50 of the 326 variants (Supplementary Table 10). The classification of the 26 variants deemed to be pathogenic/likely pathogenic was driven by absence from the gnomAD database (92%, P6), computational evidence (88%, P9), functional data (73%, P3) and *de novo* status (50%, P2). The classification of the 55 variants classified as benign was largely driven by variant frequency in gnomAD exceeding the maximum tolerated allele count (100%, B2) and only one or none of the computational prediction tools indicating pathogenicity (11%, B5).

Reason for referral and genetic testing outcome

Cases referred for genetic testing at MCGM with a more confident diagnosis of CPVT based on clinical evaluation were tested using the CPVT genetic panel, whereas those cases with less diagnostic certainty were tested using either the arrythmia panel (including 37 genes associated with inherited arrhythmia) or the molecular autopsy panel (61 genes associated with sudden cardiac death). The proportion of patients referred for genetic testing with the CPVT panel that carried *RYR2* variants was significantly greater than that of the patients tested with the arrhythmia panel ($P < 0.05$) or molecular autopsy panel ($P < 0.0005$). Furthermore, these patients were more likely to carry pathogenic *RYR2* variants ($P < 0.05$) (Table 4, Figure 3).

Discussion

The availability of sequence variant databases like gnomAD (11) and a statistical threshold to aid in the classification of pathogenicity for genetic variants (16) is aiding the robust classification of

sequence variants as associated, or not, with disease. The maximum tolerated allele count method was validated in individuals with hypertrophic cardiomyopathy using previous variant assessments and reports of pathogenicity in ClinVar (14). In the present study we used this method to calculate a maximum tolerated allele count for CPVT-associated *RYR2* missense variants. Using this frequency threshold, 55 of 326 (16.9%) CPVT associated *RYR2* variants were re-classified as benign. Thus, our data show a sizeable number of *RYR2* variants are not disease-causing, in which case the proportion of CPVT cases attributable to *RYR2* variants is likely to be over-estimated and the proportion of cases attributable to changes in other genes or to post-translational modifications is likely to be underestimated. The reclassification of VUS as benign variants is important as family members previously cascade tested to carry these variants may not be at increased risk and those without these variants may have been falsely reassured and remain at risk of arrhythmia or sudden cardiac death. Furthermore, this classification of benign variants offers the opportunity to find the real explanation for the CPVT phenotype in affected individuals.

In the present study the maximum tolerated allele count for CPVT associated *RYR2* missense variants was calculated using both the ExAC and gnomAD databases as control populations. Variants that occurred above the frequency threshold in each population where then reclassified accordingly. The ExAC database contains exome data from 60,706 unrelated apparently healthy individuals, whereas the gnomAD database contains combined exome and genome variant data from 141,456 individuals. Importantly 1600 of the 1975 (81%) *RYR2* missense variants reported in gnomAD have a minor allele count below four. This not only highlights that a number of potentially healthy individuals have rare variants in *RYR2* which may have a consequence in the context of a particular trigger e.g. exercise or emotion, but also that many benign *RYR2* variants are rare. The utility of larger control datasets in reclassifying *RYR2* variants was exemplified in this study. Comparison with the larger gnomAD database as a control population allowed the reclassification of 11 additional *RYR2* variants as benign compared to ExAC. Further reclassification of VUSs may be achieved with larger sequence

datasets and by using data from individuals with more phenotype data and of older age to reduce the effects of non-penetrance.

Applying Consurf, we found *RYR2* variants present in CPVT patients occurred at amino acid positions that were significantly more conserved than those of control variants and the conservation of residues where CPVT sudden death variants occurred was even greater (Figure 2). The application of a frequency threshold in control datasets with the consideration of Consurf may be more informative than using each method independently and may be particularly useful for determining the probability of a rare variant being pathogenic or benign.

Both *RYR2* variants and CPVT are commonly associated with arrhythmias and/or sudden death triggered by exercise or stress. However, we noted *RYR2* variants in individuals who died or experienced cardiac arrest while asleep. In these patients almost all of the *RYR2* variants that were not classified as benign resulted in changes within the C-terminal of the protein, with the exception of one variant that occurred in the central domain. Although, limited by the small number of cases this data suggests pathogenic C-terminal *RYR2* variants may pose a greater risk of sudden death at rest, particularly during sleep. This relationship between C- terminal variants and sudden death in sleep is novel and requires independent validation. Sleep is considered a restful period but during rapid eye movement (REM) sleep, which accounts for approximately 20% of sleep time, sympathetic activity is increased and intense emotional states occur (19). Thus, sudden death during sleep in patients with *RYR2* variants may be due to episodes triggered by increased sympathetic activity comparable to exercise or emotional stress. Contrastingly, specific *RYR2* variants may exhibit properties that increase their sensitivity to other sleep related triggers like rises in hormones such as melatonin which has been shown to induce ventricular arrythmias (20, 21). Importantly in a recent prospective study of sudden cardiac death the majority of deaths occurred during sleep (22) and CPVT should be considered as a potential cause in this setting.

Our data shows that CPVT-associated *RYR2* variants predominantly cluster in four regions/domains, namely the RyR and IP3R homology-associated domain; the ion transport domain; and two regions outside of known domains (No domain regions 5 and 7). Generally, these regions correspond to the previously-reported mutation hotspots. However, more than 30% of CPVT-associated *RYR2* variants occurred outside of mutation hotspots, emphasising the importance of screening the entire coding region of *RYR2* in patients suspected of having CPVT.

The presence of functional data was a major driver of pathogenic classifications. However functional data was only available for 50 of the 326 CPVT associated variants. In addition to this the threshold of at least a 50% effect on channel function required for pathogenicity as applied by Denham et al. (2019) may not be applicable for *RYR2* as there is no direct correlation between the magnitude of variant functional effect and disease phenotype in CPVT (15). Computational evidence and absence from control datasets were also major contributors to pathogenic classifications, similar to Denham et al. (2019), we used eight computational tools and applied a threshold of six tools predicting a pathogenic effect for pathogenic classification (15). This method was found to be more stringent and require more evidence for a pathogenic classification when compared to previous systems (15).

A limitation of this study was the lack of systematically collected phenotype data and this will be required prospectively to identify effective means of combining clinical and genetic information to make accurate CPVT diagnoses. Nonetheless, based on the clinical indications considered our data shows specificity of testing (a surrogate for confidence in the underlying phenotype) correlates with the likelihood of identifying a relevant variant. Thus, although genetic testing is a useful aid in the diagnosis of CPVT rigorous clinical evaluations and the establishment of additional common phenotypic traits for CPVT is likely to increase the efficiency of genetic testing, identification of pathogenic variants and possibly improve the management of the condition.

In summary, CPVT-associated *RYR2* variants cluster in specific domains/regions, many of which are within, but not confined to, previously established mutation hotspots. CPVT-associated variants

occur at residues that are more evolutionarily conserved than controls, and *RYR2* variants associated with sudden death occur at positions which are even more highly conserved. The application of a frequency threshold for pathogenicity, amino acid conservation scores and functional data aid distinguishing pathogenic and benign variants. However, the majority of CPVT variants remain classified as VUS. Therefore, additional approaches are required, including sharing of sequence data from affected individuals through Clinvar and other resources, generation of additional sequence data from healthy controls and use of sensitive high-throughput functional assays like saturation genome editing, with sufficient weight to drive pathogenic or benign classifications (23).

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Supplementary information is available at the Journal of Human Genetics website.

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405 Titles and legends to figures

406 Figure 1A. The distribution of missense variants in RYR2 in control population from the gnomAD
407 database (A), CPVT (B), sudden death (C) and sudden death in sleep (D) populations.

408

409 Figure 1B. Proportion of RYR2 variants in grouped domains in controls from the gnomAD database,
410 CPVT, sudden death and sleep-associated sudden death populations. The number of cases in the
411 sudden death and sleep-associated sudden death groups was limited, there were 47 sudden death
412 cases and 7 sleep-associated sudden death cases.

413

414 Figure 1C. Grouped domains of RYR2 in which the proportion of RYR2 variants was significantly
415 different in controls from gnomAD compared to CPVT patients. **** and *** represent $P < 0.0001$
416 and $P < 0.005$, respectively.

417

418 Figure 2. A) Consurf scores of amino acid positions of CPVT variants compared to controls from
419 gnomAD. B) Consurf scores of amino acid positions of non-sudden death CPVT variants compared to
420 sudden death CPVT variants. **** represents $P < 0.0001$.

421

422 Figure 3. A) Number of patients referred for genetic testing using the CPVT, arrhythmia or molecular
423 autopsy panel with an RYR2 variant detected. B) Number of patients referred for genetic testing
424 using the CPVT, arrhythmia or molecular autopsy panel with a pathogenic RYR2 variant detected.

Table 1. Pre-established RyR2 variant hotspot regions in CPVT.

Variant hotspot region	**Residues (amino acids)**	**variants (% of total) n=326**	***de novo* variants (%) n=40**	**Male:Female Ratio (%)**
I	77-466	35 (10.7)	7 (17.5)	56:44
II	2246-2534	48 (14.7)	6 (15)	50:50
III	3778-4201	70 (21.5)	13 (32.5)	42:58
IV	4497-4959	69 (21.2)	10 (25)	41:59
Non-hotspot regions		104 (31.9)	4 (10)	47:53

Table 2. Proportion of *RYR2* variants in individual *RYR2* domains or regions in controls from gnomAD, CPVT, sudden death and sleep-associated sudden death populations.

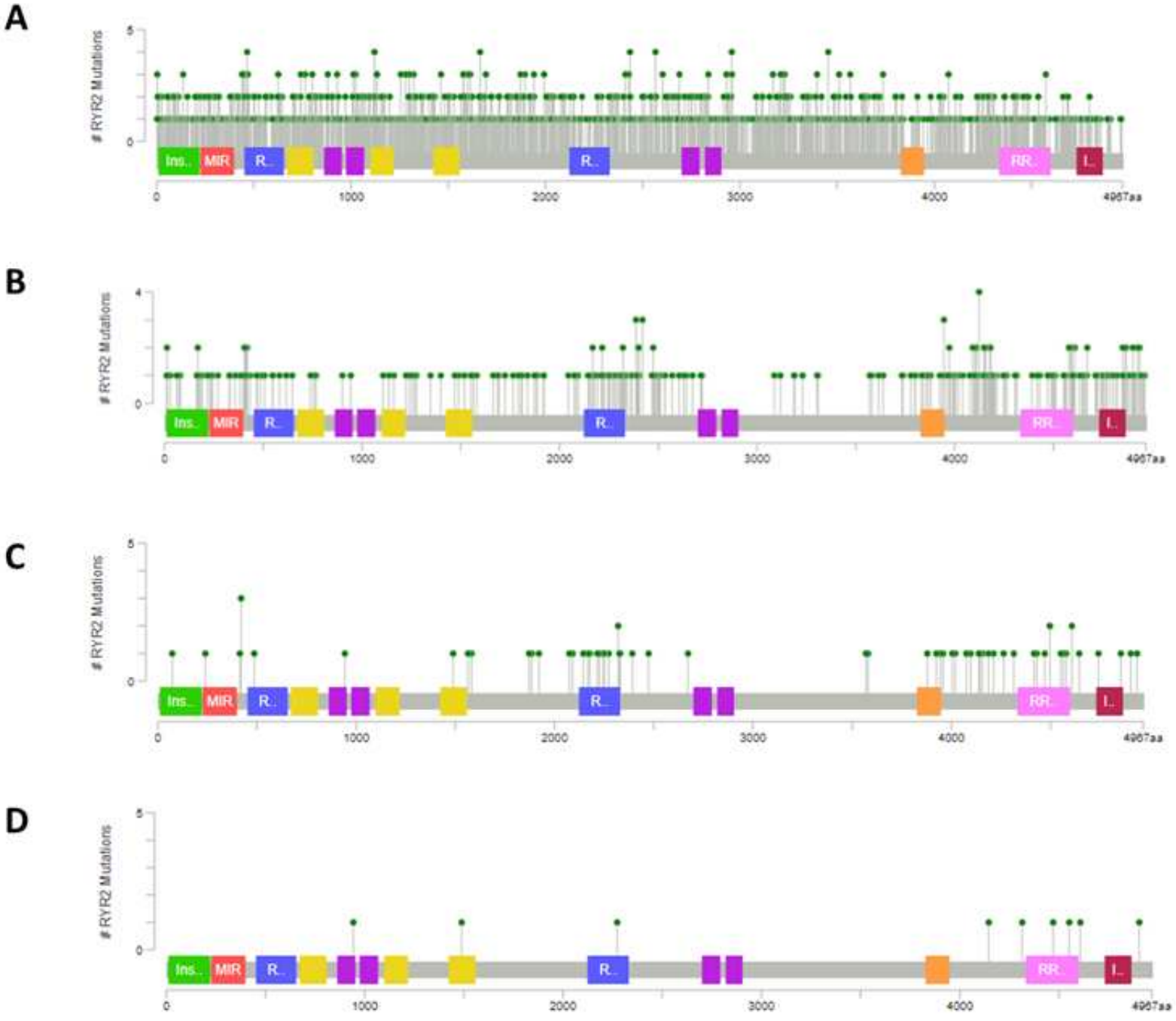
Protein domain or region	Length of region (delimiting amino acids)	Control population from gnomAD database (%)	CPVT (%)	Sudden death (%)	Sleep (%)
Inositol 1,4,5-trisphosphate/ryanodine receptor	212 (10-222)	100 (5.1)	18 (5.5)	1 (1.6)	0
MIR domain	173 (226-399)	78 (3.9)	9 (2.8)	1 (1.6)	0
No domain #1	51 (400-451)	31 (1.6)	15 (4.6)	4 (6.3)	0
RYDR_ITPR domain #1	203 (452-655)	89 (4.5)	9 (2.8)	1 (1.6)	0
SPRY domain #1	137 (671-808)	75 (3.8)	4 (1.2)	0	0
RyR domain #1	91 (862-953)	39 (2.0)	2 (0.6)	1 (1.6)	1 (11.1)
RyR domain #2	93 (975 - 1068)	46 (2.3)	0	0	0
SPRY domain #2	120 (1099 - 1219)	60 (3.0)	3 (0.9)	0	0
SPRY domain #3	135 (1424 - 1559)	59 (3.0)	4 (1.2)	1 (1.6)	1 (11.1)
No domain #2	564 (1560-2122)	282 (14.3)	21 (6.4)	5 (7.9)	0
RYDR_ITPR domain #2	208 (2123 - 2331)	52 (2.6)	29 (8.9)	12 (19)	1 (11.1)
No domain #3	367 (2332-2699)	160 (8.1)	38 (11.7)	3 (4.7)	0
RyR domain #3	93 (2700 - 2793)	34 (1.7)	3 (0.9)	0	0
RyR domain #4	85 (2820 - 2905)	26 (1.3)	0	0	0
No domain #4	920 (2906-3826)	352 (17.8)	16 (4.9)	2 (3.2)	0
RyR and IP3R Homology associated	121 (3827 - 3948)	19 (1.0)	11 (3.4)	4 (6.3)	0
No domain #5	383 (3949-4332)	133 (6.7)	64 (19.6)	10 (15.9)	1 (11.1)
Ryanodine Receptor TM 4-6	266 (4333 - 4599)	96 (4.9)	20 (6.1)	8 (12.7)	2 (22.2)
No domain #6	130 (4600-4730)	38 (1.9)	16 (4.9)	3 (4.7)	2 (22.2)
Ion_transport domain	135 (4731 - 4866)	19 (1.0)	18 (5.5)	2 (3.2)	0
No domain #7	100 (4867-4967)	10 (0.5)	21 (6.4)	1 (1.6)	1 (11.1)

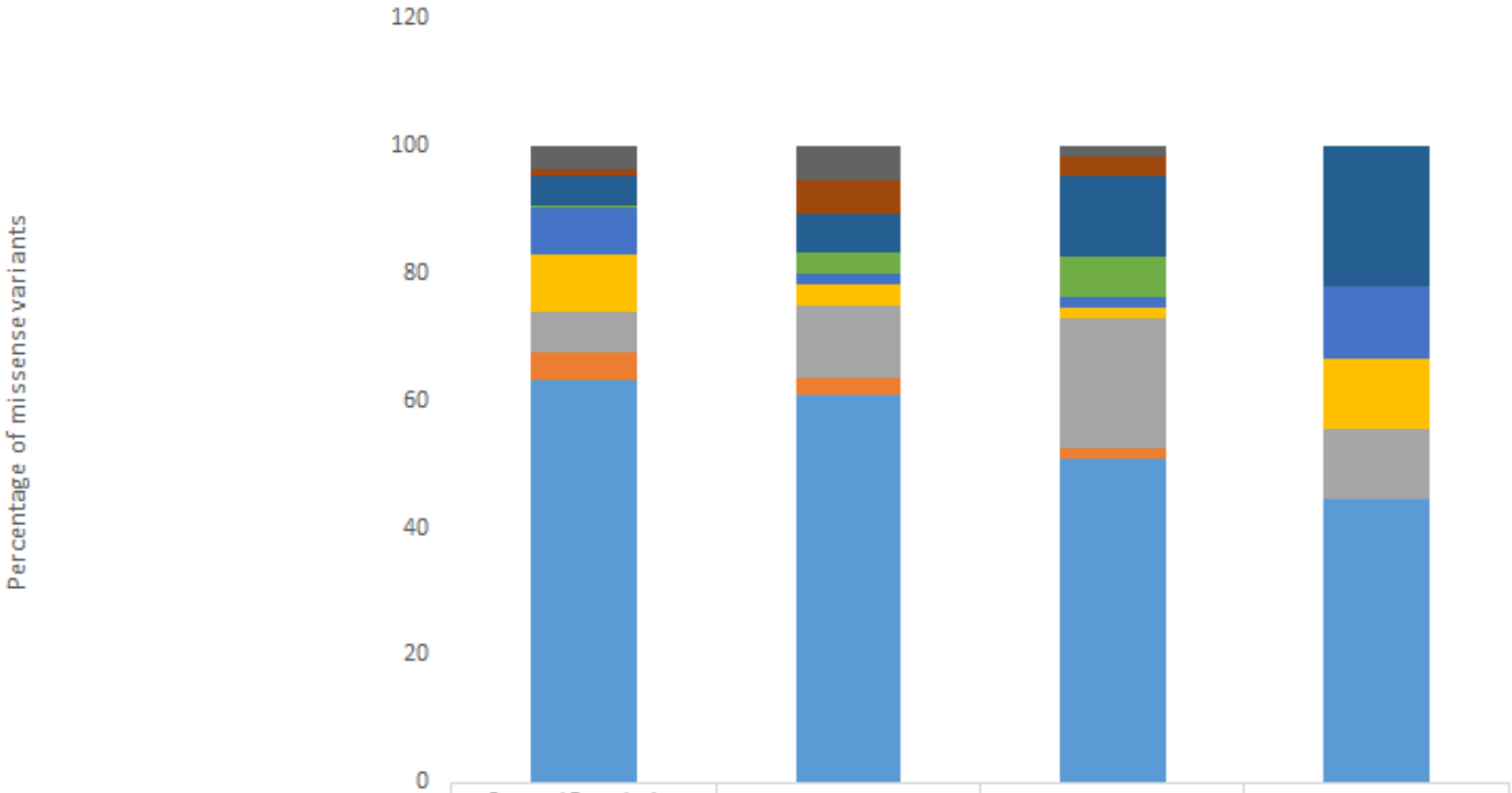
Table 3. *RYR2* variant classification based on the ACMG-AMP guidelines

ACMG classification	Number of CPVT associated RYR2 variants total = 326 (%)
Benign	55 (16.9)
Variant of uncertain significance	245 (75.6)
Likely pathogenic	14 (4.1)
Pathogenic	12 (3.7)

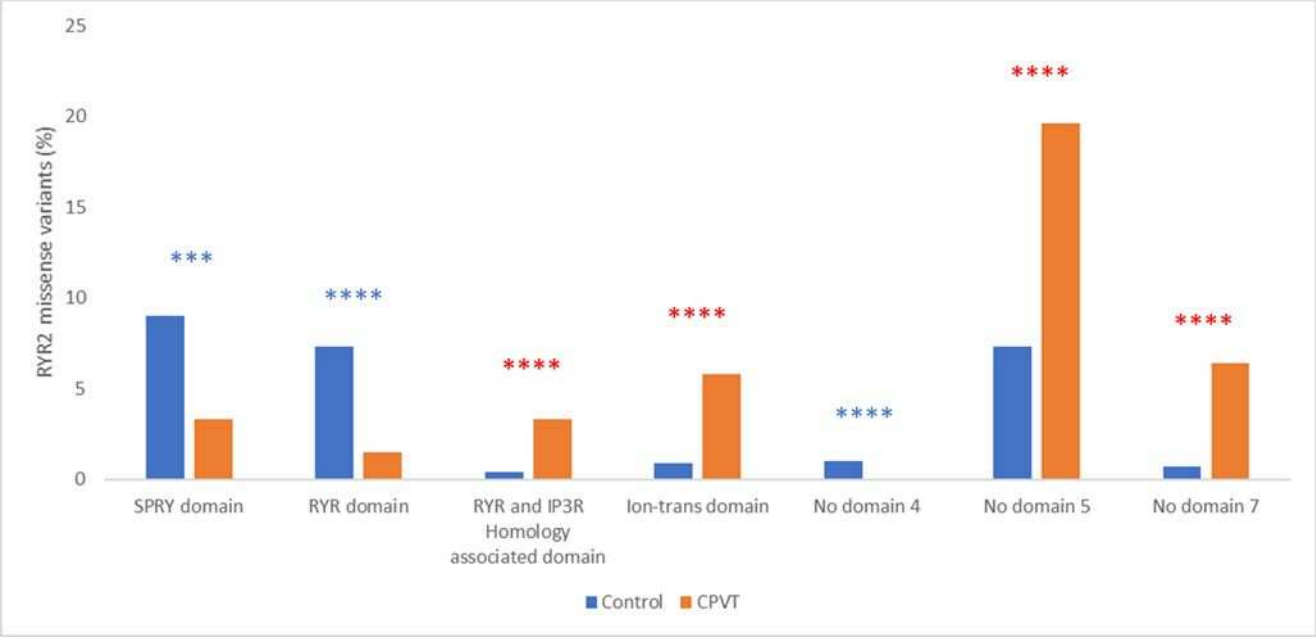
Table 4. Outcome of genetic testing for patients referred for CPVT, arrhythmia and molecular autopsy panels to the Manchester Laboratory (MCGM).

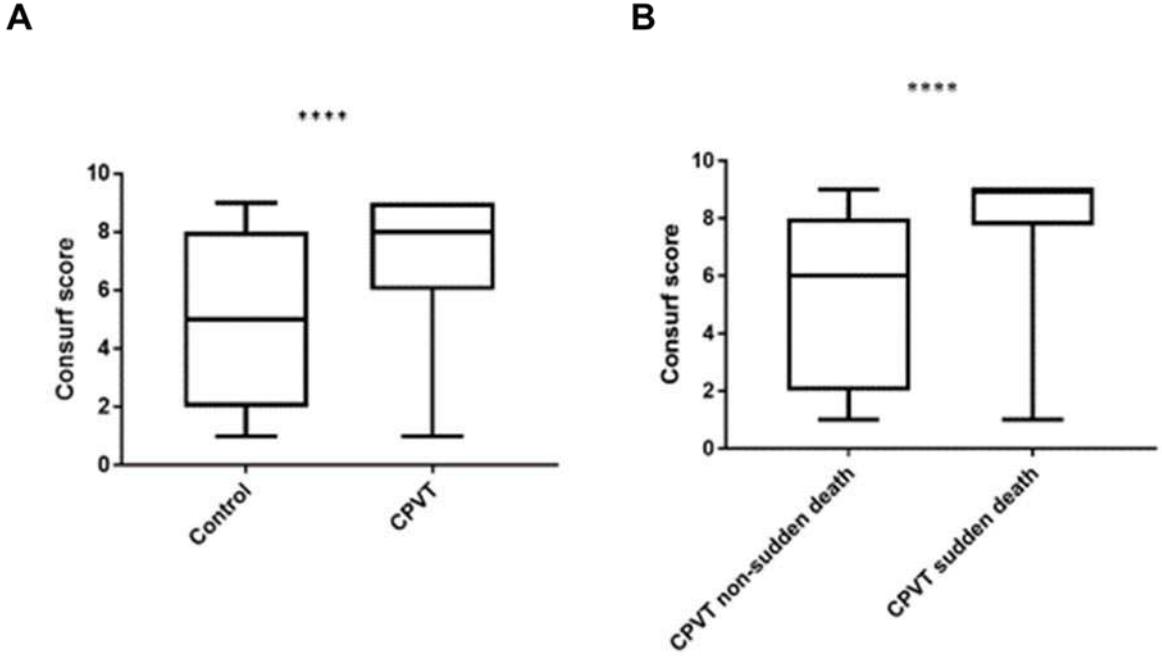
	CPVT panel	Arrhythmia panel	Molecular autopsy panel
Patients tested	98	130	166
Patients with RYR2 variants	20	11	8
Patients with pathogenic RYR2 variants	10	2	1

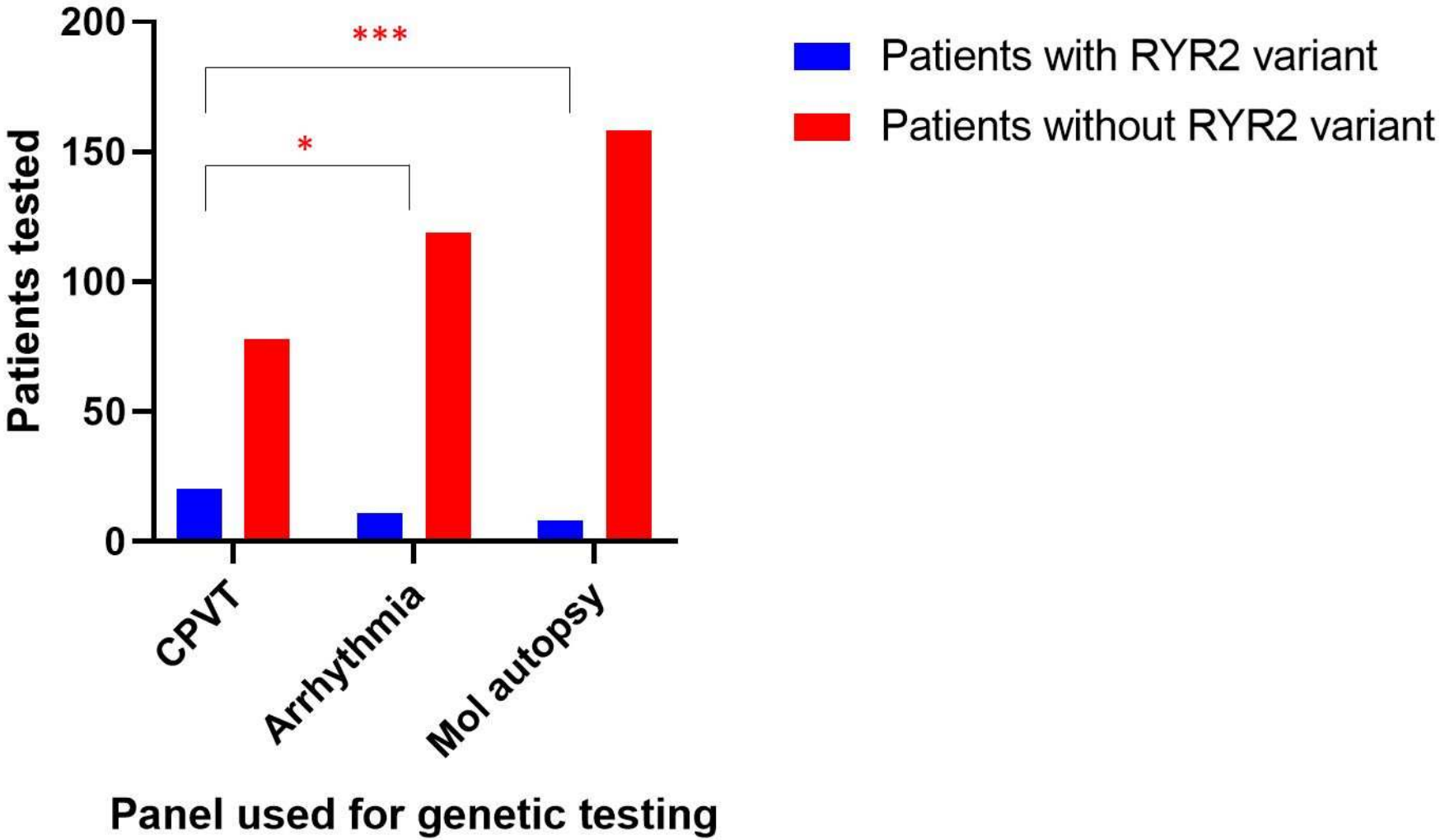

A
RYR2 Mutations
5
0
Ins..
MIR
R..
R..
RR..
I..
0
1000
2000
3000
4000
4967aa
B
RYR2 Mutations
4
0
Ins..
MIR
R..
R..
RR..
I..
0
1000
2000
3000
4000
4967aa
C
RYR2 Mutations
5
0
Ins..
MIR
R..
R..
RR..
I..
0
1000
2000
3000
4000
4967aa
D
RYR2 Mutations
5
0
Ins..
MIR
R..
R..
RR..
I..
0
1000
2000
3000
4000
4967aa

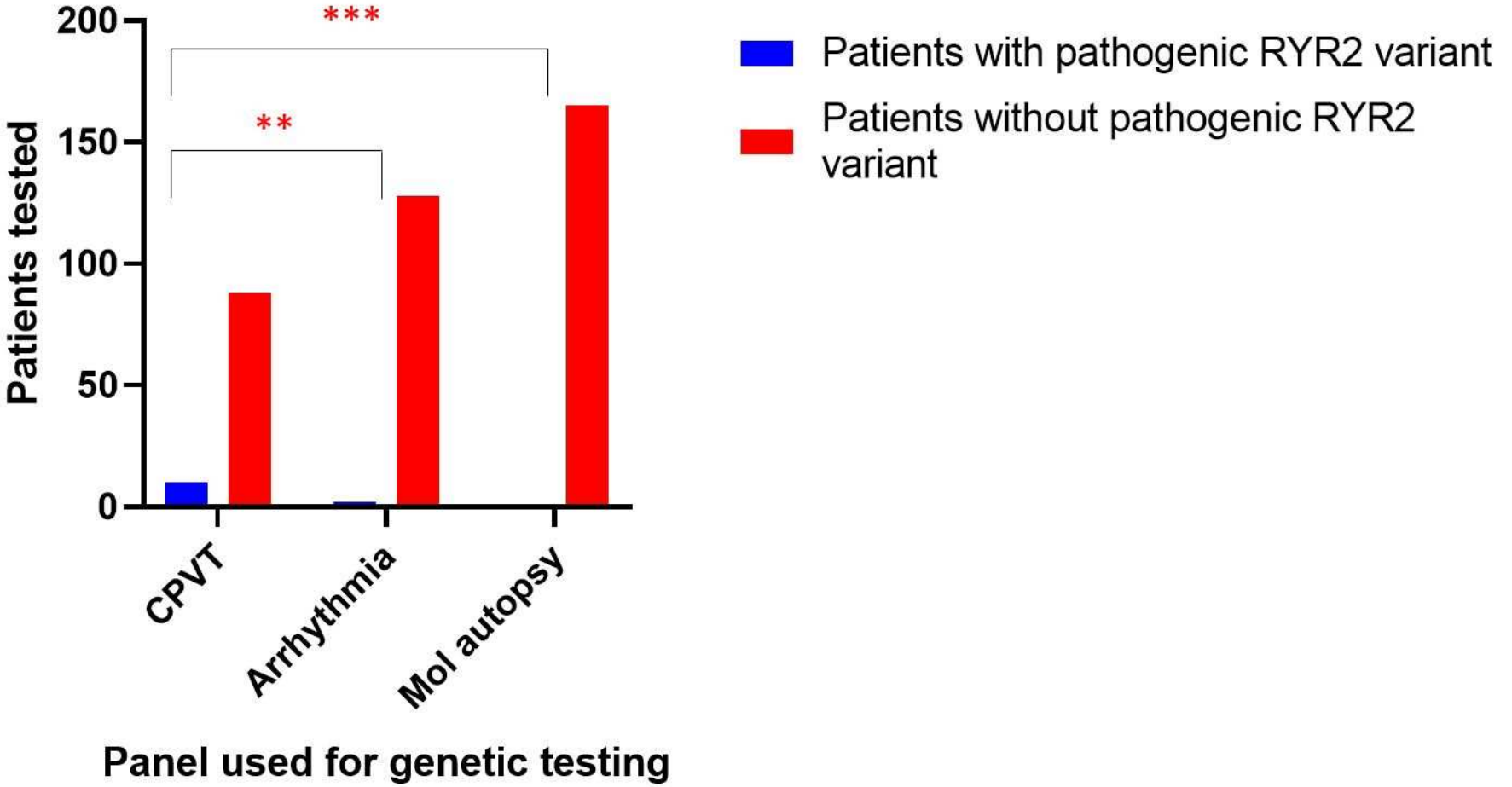


	Control Population (%)	CPVT (%)	Sudden death (%)	Sleep (%)
Inositol 1,4,5-trisphosphate/ryanodine receptor	3.8	5.2	1.6	
Ion_trans domain	0.9	5.5	3.2	
Ryanodine Receptor TM 4-6	4.5	6.1	12.7	22.2
RyR and IP3R Homology associated	0.4	3.4	6.3	
RyR domain	7.3	1.5	1.6	11.1
SPRY domain	9	3.3	1.6	11.1
RYDR_ITPR domain	6.4	11.4	20.6	11.1
MIR domain	4.4	2.8	1.6	
No domain	63.3	60.8	50.8	44.5


25
20
15
10
5
0
RYR2 missense variants (%)
***
****
****
****
****
****
****
SPRY domain
RYR domain
RYR and IP3R Homology associated domain
Ion-trans domain
No domain 4
No domain 5
No domain 7
Control
CPVT


A
****
Consurf score
10
8
6
4
2
0
Control
CPVT
B
****
Consurf score
10
8
6
4
2
0
CPVT non-sudden death
CPVT sudden death


200
150
100
50
0
Patients tested
***
*
CPVT
Arrhythmia
MoI autopsy
Panel used for genetic testing
Patients with RYR2 variant
Patients without RYR2 variant


***
**
200
150
100
50
0
Patients tested
CPVT
Arrhythmia
MoI autopsy
Panel used for genetic testing
Patients with pathogenic RYR2 variant
Patients without pathogenic RYR2 variant